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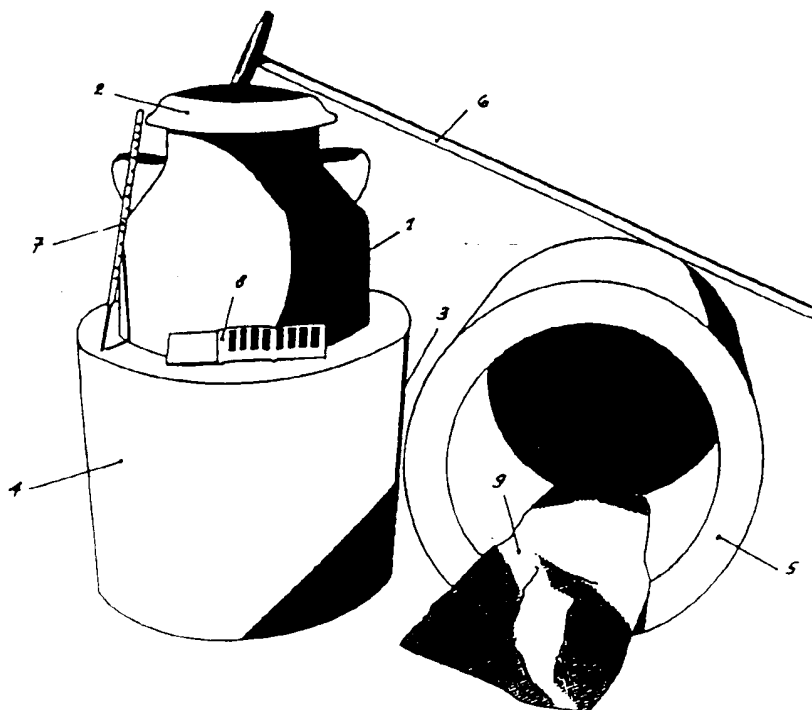
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification 5 : C12M 1/00</p>	<p>A1</p>	<p>(11) International Publication Number: WO 90/02167 (43) International Publication Date: 8 March 1990 (08.03.90)</p>
<p>(21) International Application Number: PCT/EP89/00930 (22) International Filing Date: 7 August 1989 (07.08.89) (30) Priority data: 21731 A/88 23 August 1988 (23.08.88) IT (71) Applicant (for all designated States except US): ANIDRAL S.R.L. [IT/IT]; Via Marconi, 3/B, I-28100 Novara (IT). (72) Inventor; and (75) Inventor/Applicant (for US only): MOGNA, Giovanni [IT/IT]; Via Marconi, 3/B, I-28100 Novara (IT). (74) Agent: BIANCHETTI, Giuseppe; Studio Consulenza Brevettuale, Via Rossini, 8, I-20122 Milano (IT).</p>		<p>(81) Designated States: AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CF (OAPI patent), CG (OAPI patent), CH (European patent), CM (OAPI patent), DE (European patent), DK, FI, FR (European patent), GA (OAPI patent), GB (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL (European patent), NO, RO, SD, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US. Published With international search report.</p>

(54) Title: KIT FOR THE REACTIVATION OF DEHYDRATED MICROBIAL CULTURES, PARTICULARLY OF BACTERIA

(57) Abstract

A kit comprises a stiff container (1) in plastic material, preferably polythene, with the corresponding cap (2), a thermosealed protective film, sterilized by gamma rays, an inertial thermostat (3) in foamed material, a stirrer (6) in tear-proof plastic material, and optionally a thermometer (7) and a pH measurement paper strip (8) and an anhydrous dose of a specific microbial culture (9) is described.



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KIT FOR THE REACTIVATION OF DEHYDRATED MICROBIAL
CULTURES, PARTICULARLY OF BACTERIA

The present invention refers to a kit for the re-activation of dehydrated microbial cultures, particularly of bacteria.

5 The reactivation of a bacterial culture which has been dehydrated for conservation purposes is necessary to allow the full metabolic functionality of the microorganisms and particularly to guarantee an high fermentative ability for the final substrates thereof.

10 In order to obtain satisfactory results, it is necessary that the preparation is carried out in conditions of absolute bacteriologic sterility and at constant temperature for a prefixed time, the better parameters being usually reported on the product packaging and changing according to formulations.

15 Usually, the commercial anhydrous microbial cultures are reconstituted, i.e. brought again to the liquid status, either by simply suspending them in water or by directly adding them to the substrate which they must ferment.

20 These operations are carried out in somewhat uncomfortable conditions by means of apparatuses not specifically designed for the intended use, with consequent difficulties in securing the above said conditions, with a consequent low yield in the use thereof.

25 The object of the invention is to avoid the above said drawbacks by providing a kit for the reactivation of

dehydrated microbial cultures, allowing the operations to be carried out in conditions of absolute sterility and the maintenance of a perfect constance of the temperature.

5 The kit according to the invention substantially comprises a stiff container in plastic material, preferably polythene, with the corresponding cap, a thermosealed protective film, preferably of polythene for
10 alimentary use, sterilized by gamma rays, an inertial thermostat in foamed material, for instance of foamed polystyrene, a stirrer in tear-proof plastic material, for instance of PVC, and optionally a thermometer and a pH measurement paper strip and an anhydrous dose of a specific microbial culture.

15 The thermostat in foamed material preferably comprises an envelope containing the stiff container and it is divided in two halves.

~~Said~~ kit is extremely simple and economic and it may be used by untrained personell.

20 The reactivation of the microbial culture by means of the kit of the invention is carried out in the following way:

- the stiff container is inserted in one of the two halves of the thermostat in foamed material;
- 25 - one of the two thermosealed edges of the sterile polythene bag is cut and the open bag is inserted in the stiff container with the opening towards the upper side;
- about 30 liters of warm (about 30-35°C) tap water are
30 introduced in the bag;

- the content of an anhydrous dose is poured therein;
- the mixture is stirred up to the complete dissolution of the powder;
- further tap water is added up to the container edge, checking the temperature by means of the thermometer;
- the bag is closed, for instance by means of a rubber band and the container by its cap;
- the other half of the thermostat in foamed material is placed so as to hold the container, keeping the kit in these conditions for the specified time;
- the correct pH value is checked by means of the pH measurement paper strip.

A preferred embodiment of the kit for the reactivation of microbial cultures according to the invention will be hereinafter described with reference to the annexed figure, showing in perspective view the different components.

With reference to said figure, the kit according to the invention comprises a stiff container 1, preferably in polythene, substantially cylindrical, with the corresponding cap 2, an inertial thermostat in foamed polystyrene 3, forming a covering envelope of the container 1 and divided in two identical halves 4 and 5, a rod stirred 6, a thermometer 7, a pH measuring paper strip 9 and a thermosealed protective film for alimentary use, sterilized by gamma rays, which is not shown in the enclosed figure.

According to a preferred embodiment, the stirrer consists in an holed washer having along its circumference a groove provided to contain an O-ring in

soft material, acting as shock absorber, the washer itself being fixed into a rod having length so as to prevent the direct contact of the washer with the bottom of the container, so as to avoid the tearing of the polythene bag during stirring.

The reactivation of the microbial culture using the kit according to the invention is carried out in the following way:

- the stiff container 1 is inserted into one of the two halves of the thermostatic envelope 3;
- one of the two thermosealed edge of the sterile polythene bag (not shown) is cut and inserted into the stiff container 1 with the opening toward the upper side;
- a certain amount of warm tap water (about 30 l at a temperature of about 30-35°C) is introduced into the container 1;
- the anhydrous dose 9 is introduced as a powder into the container 1;
- the mixture is stirred by the stirrer 6 up to complete dissolution of the powder;
- further tap water is added up to the container upper edge, checking the temperature of the mixture by the thermometer 7;
- the sterile bag is closed, for instance by means of a rubber band, and the cap 2 is applied on the container 1;
- the other half of the polystyrene thermostatic envelope is inserted, and the whole kit is kept in these conditions for the time specified in the instructions

enclosed to the different kinds of cultures.

The correct pH value is checked by means of the pH measurement strip.

5 - When the reactivation is ended, the product is ready to use.

By means of the kit of the invention, allowing to work in conditions of absolute sterility and at perfectly constant temperature, even in environments where it is not normally possible to secure these conditions, 10 excellent results are obtained in the reactivation process of microbial cultures; the microorganisms turn out to be much more active and numerically increased so as to allow working yields even ten times higher with the same dose of used microbial culture.

15 It should be pointed out that the apparatuses comprising the kit are particularly cheap and easy to use also by untrained personell.

Of course the invention is not limited to the particular embodiment above described and shown in the 20 enclosed figure, and several changes may be made to it without departing from the scope of the invention.

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CLAIMS

1. A kit for the reactivation of dehydrated microbial culture, particularly of bacteria, comprising:
 - 5 - a stiff container ~~1~~ with the corresponding cap (2);
 - a thermosealed protective film, sterilized by gamma rays, suited to coat the inner walls of the container (1) during the use;
 - an envelope ~~in~~ foamed material (3) covering the
10 container (1) and acting as an inertial thermostat;
 - an anhydrous dose of a specific microbial culture (9) to be mixed with water in the container (1);
 - a rod stirrer (6), and optionally a thermometer (7) and pH measuring paper strips (8).
- 15 2. A kit according to claim 1, characterized in that the thermostatic envelope (3) consists of two halves (4 and 5).
3. A kit according to claim 1, characterized in that said container (1) is of cylindrical shape.
- 20 4. A kit according to any one of claims 1 to 3, characterized in that the container (1), the corresponding cap (2) and the protective film are made of polyethylene and the stirrer (6) is made of PVC.
5. A kit according to any one oc claims 1-4,
25 characterized in that the envelope acting as inertial thermostat is made of foamed polystyrene.
6. A kit according to claims 1-5, characterized in that the stirrer consists of an holed washer having along its circumference a groove having an O-ring of soft
30 material, said washer being fixed onto a rod having a

length so as to avoid the direct contact of the washer
with the bottom of the container.

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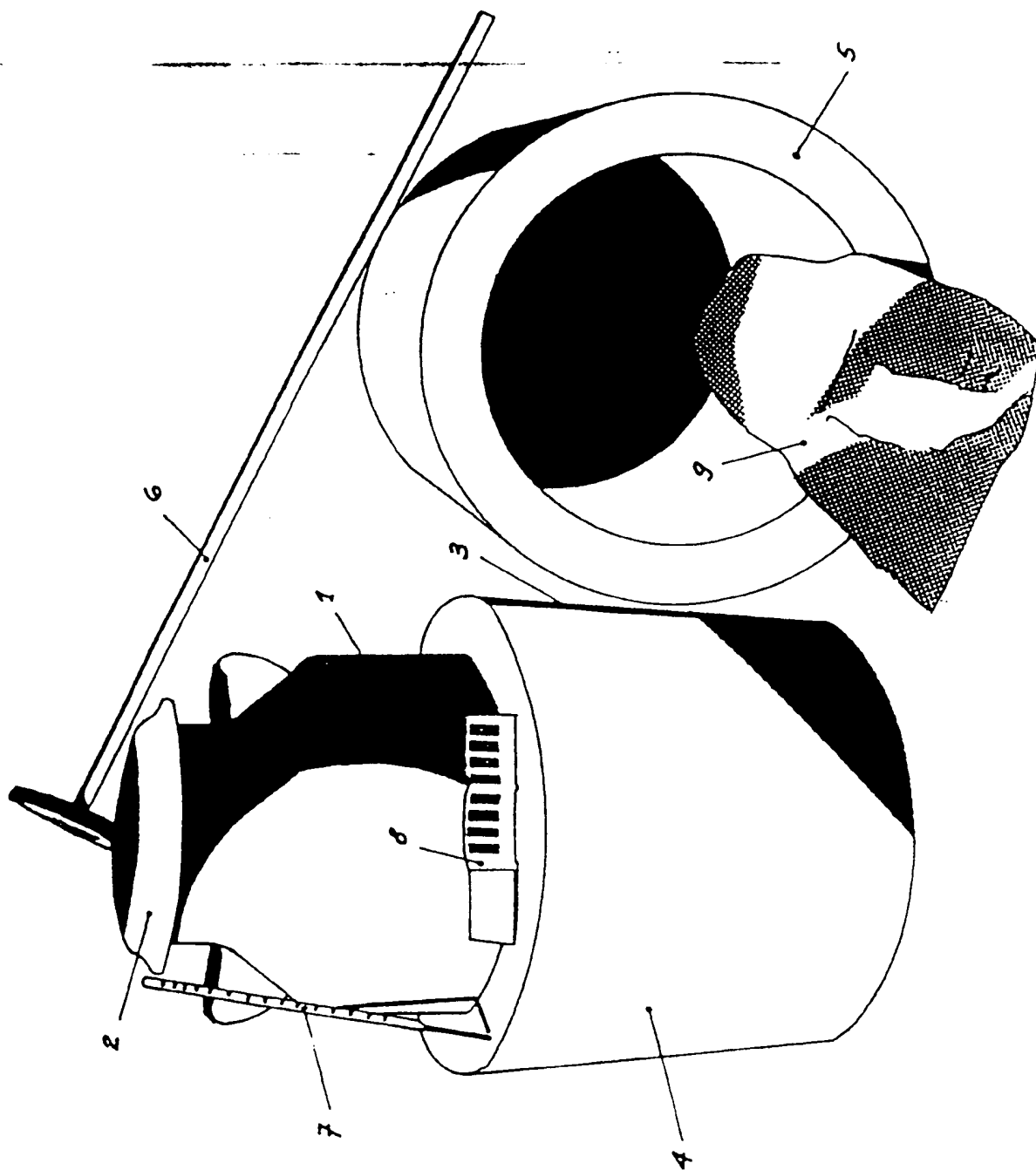
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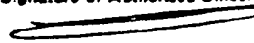
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INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 89/00930

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC ⁵ : C 12 M 1/00		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC ⁵	C 12 M, C 12 G	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	FR, A, 2296010 (SPOFA USINES PHARMACEUTIQUES REUNIES) 23 July 1976 see claims 1,2,3; page 2, column 1 --	1,4
A	FR, A, 1078976 (L. THIES) 19 May 1954 see page 2, column 2; page 3, column 1; figure --	1,3
A	GB, A, 1299075 (NATIONAL RESEARCH DEVELOPMENT) 6 December 1972, see figure 1; claims; page 1, lines 62-78 --	1,4
A	FR, A, 1389044 (S.B. PENICK et al.) 4 January 1965 --	
A	FR, A, 1601395 (F. SERRES) 17 August 1970 see figure; abstract --	1,5
E	GB, A, 2202549 (P.J. WHITNEY) 28 September 1988 see figure 4, claims --	1
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"A" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
15th November 1989	12.12.89	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	 T.K. WILLIS	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	FR, A, 2563531 (HICKINBOTHAM WINEMAKERS) 31 October 1985; see figure; claims -----	1,3,4,6

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

EP 8900930

SA 30246

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 06/12/89. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		GB-A- 1535199	13-12-78
		JP-A- 51088685	03-08-76
		NL-A- 7515141	01-07-76
FR-A- 1078976		None	
GB-A- 1299075	06-12-72	None	
FR-A- 1389044		None	
FR-A- 1601395	17-08-70	None	
GB-A- 2202549	28-09-88	None	
FR-A- 2563531	31-10-85	CH-B- 665651	31-05-88
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